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**Reduced soil respiration beneath invasive *Rhododendron ponticum* persists after cutting and is related to substrate quality rather than microbial community.**

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## Abstract

Invasive plants depositing recalcitrant, polyphenol-rich leaf litter may alter decomposition dynamics, leading to an accumulation of soil organic matter. Removing invasives is critical in restoring native habitats, but our understanding of its impacts upon soil processes remains limited. Here, we test the hypothesis that clearing of *Rhododendron ponticum* leads to increased soil respiration, at a site within Snowdonia National Park, Wales, UK. Soil samples were collected, and soil respiration was monitored over 32 weeks on plots cleared of *R. ponticum*, plots infested with *R. ponticum* which were left uncleared, and uninvaded plots of native vegetation. Soil respiration was significantly higher in native vegetation plots, relative to uncleared plots. Clearing *R. ponticum* led to a short-term (< four weeks) increase in soil respiration relative to uncleared plots and was related to elevated soil temperature post-clearance. However, this respiration response was transient, with no significant difference between cleared and uncleared plots over the whole growing season (32 weeks). Declining soil respiration responses to soil warming have been attributed to altered soil microbial communities and substrate limitation. Analysis of microbial phospholipid fatty acids (PLFAs) detected no differences among cleared, native and uncleared plots post-clearance. However, Fourier-transform mid-infrared spectroscopy detected a decline in organic matter aromaticity over the growing season in the native and uncleared plots, whilst there was no change in the cleared plots. The findings show that despite a pulse in soil respiration during the initial four weeks post-clearance, *R. ponticum* litter and associated soil organic matter in cleared plots continued to decompose at a similar rate to uncleared plots over the whole growing season. This was likely a result of substrate limitation and altered soil organic matter composition following *R. ponticum* clearing, with labile carbon becoming depleted and an enrichment of more recalcitrant aromatic structures.

**Keywords:** “Invasive”; “soil”; “respiration”; “decomposition”; “PLFAs”; “FTIR”; “soil organic matter”.

## 1. Introduction

Invasive plants can alter soil processes such as soil organic matter (SOM) decomposition by depositing high quantities of chemically distinct litter (Suseela et al., 2016; Tamura et al., 2017). The decomposition of leaf litter is determined by three main factors: litter chemical composition, soil physicochemical properties and the microbial community (Jewell et al., 2015). Plant invasions have the potential to alter all three of these factors, with litter chemistry particularly important (Pattison et al., 2016; Suseela et al., 2016; Tamura et al., 2017). Plants adapted for high-nutrient environments produce labile litter which rapidly decomposes and leads to lower soil carbon content post-invasion (Liao et al., 2008; Tamura and Tharayil, 2014). Conversely, plants adapted for low-nutrient environments produce low-quality litter, which is rich in phenolic compounds (Aerts, 1999; DeLuca et al., 2013; Hobbie, 1992). This recalcitrant litter decomposes slowly, leading to altered soil microbial communities, shifts in SOM composition and increased carbon sequestration (Ehrenfeld et al., 2001; Suseela et al., 2016; Tamura and Tharayil, 2014).

Altering soil processes may be important for certain invasive shrubs, such as the UK invasive plant *Rhododendron ponticum* L., given that ericaceous species leaf litter strongly influences decomposition dynamics (Aerts, 1997; DeLuca et al., 2013). It is known that related *Rhododendron* spp. produce acidic, polyphenol rich litter which decomposes slowly (Monk et al., 2014; Wurzbürger and Hendrick, 2007). Furthermore, tannins leaching from the litter of *Rhododendron* spp. can inhibit microbial activity, form decay-resistant complexes with proteins and inactivate enzymes in the underlying soil (Hättenschwiler and Vitousek, 2000; Horner et al., 1988; Wurzbürger and Hendrick, 2009). The slow decomposition of *Rhododendron* spp. litter results in the formation of a thick layer of undecomposed litter on the soil surface (Plocher and Carvell, 1987) and an accumulation of SOM (Wurzbürger and Hendrick, 2007).

Whilst many studies have observed altered decomposition dynamics beneath invasive plants, few have looked at how clearing invaded sites affects soil processes (Frank et al., 2018; Osburn et al.,

2018). Recent years have seen a considerable effort to control the spread of invasives such as *R. ponticum* and restore native habitats in the UK (Jackson, 2008; Snowdonia Rhododendron Partnership, 2015; Tyler et al., 2006). The most common method of control for large patches of mature *R. ponticum* thickets is to cut and either burn or chip the stems, whilst periodically revisiting sites to apply herbicide on *R. ponticum* regrowth (Edwards, 2006; Jackson, 2008). This method of controlling invasives exposes large areas of bare ground, potentially influencing soil functioning and properties in many ways.

Firstly, the bare ground is exposed to increased solar radiation following canopy removal (Araujo and Austin, 2015). This would lead to increased soil temperature, and in turn, higher decomposition rates through enhanced microbial activity (Eliasson et al., 2005; Hartley et al., 2007; Rutledge et al., 2010). Increased solar radiation may also lead to greater photochemical breakdown of the recalcitrant litter compounds such as lignin and tannins, further enhancing the release of CO<sub>2</sub> from soil (Austin et al., 2016; Gliksman et al., 2018; Rutledge et al., 2010). Photodegradation, however, may not greatly influence soil carbon content, as UV radiation does not penetrate into the soil (Moorhead and Callaghan, 1994).

Canopy removal may also impact upon soil water content; increased surface air heat loss during the night post-clearance would lead to higher dew formation (Gliksman et al., 2018; Xiao et al., 2009) and potentially higher microbial activity as a result (Gliksman et al., 2017). Canopy interception of rainfall also results in less water reaching the ground (Llorens and Domingo, 2007), and its clearance will result in lower evapotranspiration. Removing the canopy may therefore result in higher soil moisture content, leading to higher microbial activity (Hanson et al., 2000).

Finally, cutting *R. ponticum* may change soil chemistry and microbial communities. A recent study in North America on another invasive shrub, *Lonicera maackii* (Rupr.) Herder, suggested that cutting leads to a pulse in rhizodeposition (Frank et al., 2018). Increased exudation of carbon substrates post-clearance influences the microbial community, in turn leading to altered SOM degradation

(Frank et al., 2018; Pignataro et al., 2012). Despite this, Osburn et al. (2018) found that the removal of a *Rhododendron maximum* canopy had no effect on the activity of enzymes involved in decomposition.

Invasive plant removal therefore has the potential to alter soil processes, chemical composition and microbial communities. Great emphasis is placed on removing invasive plants, given the critical role this plays in restoring native habitats and preventing further invasion (European Union, 2014). However, our understanding of how these activities influence soil processes remains limited. As it will influence the vegetation community that can inhabit the soil post-clearance, and thus the success of restoration, increasing our understanding of the impacts of clearance on soil processes is important.

This study investigated the impact of *R. ponticum* invasion and its subsequent clearance on soil functioning and chemistry, testing four hypotheses. Firstly, that soil respiration is lower on uncleared plots of *R. ponticum* relative to adjacent native vegetation plots. Secondly, that clearing the canopy increases soil respiration, relative to uncleared *R. ponticum* plots. To provide a mechanistic explanation for the above hypotheses, it was further hypothesised that *R. ponticum* clearance would alter both (iii) soil microbial communities and (iv) SOM chemical composition.

## **2. Materials and methods**

### **2.1. Site description**

A 0.9 hectare site in Tanygrisiau (52°58'55" N 3°57'18" W), Snowdonia National Park, Wales, was selected for sampling due to the presence of well-established *R. ponticum* thickets (100% *R. ponticum* cover, <3 m tall). Uninvaded areas of native vegetation cover were also present, consisting of acid grassland species typical of the area including *Agrostis capillaris* L., *Nardus stricta* L., *Molinia caerulea* L., *Juncus effusus* L., *Carex echinata* Murray and to a lesser extent, shrubs such as *Calluna vulgaris* L. (Hull) and *Vaccinium myrtillus* L.. Soil at the site was a peaty podzol of the Hexworthy series (National Soil Resources Institute, 2019). The site has a north-east facing aspect with an

average gradient of 10%. Altitude at the area sampled varied from 180 m to 190 m. On average, the site received an annual 2678 mm of rain, whilst the mean air temperature was 9.3 °C over the five years prior to sampling (Met Office, 2018).

Between the 19<sup>th</sup> and 23<sup>rd</sup> of March 2018, eight 6 m x 20 m strips were cleared of *R. ponticum* by cutting the stems at their base and burning the material off-site; roots were not removed from the soil. These cleared strips alternated with uncleared 6 m wide strips (Figure S1). Subsequently, 3m x 3m plots were placed in the middle of the alternating cleared and uncleared strips (n = 8), with each plot surrounded by a 3 m buffer strip to avoid edge effects. Additionally, eight native vegetation plots were placed where *R. ponticum* had not invaded, as close as possible to the invaded plots whilst ensuring they were not influenced by *R. ponticum*. The experiment therefore had three plot types; cleared, native and uncleared.

## **2.2. Soil respiration measurements**

Sampling was conducted before clearance on the 16<sup>th</sup> of March 2018. Five bulked soil cores (20 mm diameter) containing both O-horizon material and mineral soil were collected from each plot to a depth of 15 cm. Samples were freeze-dried (LTE Scientific Lyovac, Oldham, UK) and weighed to measure moisture content gravimetrically, then stored at -80 °C prior to chemical analyses. Soil respiration was measured on each plot between 10 am and 2 pm on three consecutive days to account for variation within timepoints, using an infra-red gas analyser (IRGA) (EGM-4, PP-systems, USA) connected to a soil chamber (SRC-1, PP-systems, USA). Alongside each soil respiration measurement, data for soil temperature (to a depth of 10 cm) (Hanson H2203A temperature probe), photosynthetically active radiation (PAR) (Skye Instruments PAR Special Sensor, Wales) and soil moisture (gravimetrically) were also collected.

To investigate the impact of *R. ponticum* canopy removal, the soil sampling and respiration measurements (and associated measurements) made at the start of the experiment were repeated six times over the following growing season (one, two, four, eight, 16- and 32-weeks post-clearance).

Soil samples collected both prior to clearance and 32-weeks post-clearance were analysed for their total carbon and nitrogen content with a Vario MAX cube analyser (Elementar, Langenselbold, Germany).

### **2.3. Phospholipid fatty acid (PLFA) analysis**

Soil microbial community structure was investigated by analysis of PLFA profiles. Freeze-dried soil samples collected from each plot, both prior to and 32 weeks post *R. ponticum* removal, were sieved to remove roots and stones (2 mm mesh) and homogenised with a ball mill (Retsch MM200, Haan, Germany). PLFAs were analysed using a high throughput method adapted from Buyer and Sasser (2012), described in full detail in the supplementary information. Briefly, PLFAs were extracted from 0.4-0.6 g of each freeze-dried soil sample using phosphate buffered Bligh and Dyer extractant. Freeze-dried extracts were subsequently dissolved in chloroform and transferred to solid phase extraction (SPE) plates (100 mg silica, Phenomenex, Torrance, CA, USA), to be eluted into a 96 well plate using acetone. Extracts were analysed using an Agilent 7890A gas chromatograph with a DB-5MS column and an FID detector.

In total, 16 PLFAs were identified in the soil samples. These included commonly used biomarkers of fungi (18:2(n-6,9), 18:2(n-6,9) and 18:1(n-9)) (Bååth and Anderson, 2003), Gram-positive bacteria (15:0i, 15:0a, 16:0i, i17:0 and a17:0) (Kidd Haack et al., 1994; Lechevalier and Lechevalier, 1988; Zelles, 1999) and Gram-negative bacteria (16:1, 7,cy-17:0, 18:1(n-7) and 7,8cy-19:0) (Galbraith and Wilkinson, 1991; Ratledge and Wilkinson, 1988; Zelles, 1999). These markers were used to measure the abundance of these microbial groups, as well as the fungi: bacteria (F:B) and Gram-positive: Gram-negative ratios (GP:GN) (Frostegård et al., 2011), which are indicators of substrate quality in soil (Fanin et al., 2019; Van Der Heijden et al., 2008).

### **2.4. Fourier-transform mid-infrared spectroscopy (FTIR) analysis**

Soil chemistry was investigated by FTIR spectroscopy. Freeze-dried soil samples collected from each plot prior to clearance and 32 weeks post-clearance were sieved and milled, as previously described



above. Spectra were measured using an IRTracer-100 spectrophotometer (Shimadzu, Japan) fitted with a Golden Gate diamond ATR accessory (Specac Ltd., Orpington, UK). Absorption was recorded in duplicate with a wavelength range of 4000 to 600  $\text{cm}^{-1}$  and resolution of 2  $\text{cm}^{-1}$ . Between each sample, a background reading was taken to ensure that any atmospheric changes in  $\text{CO}_2$  and  $\text{H}_2\text{O}$  were corrected for.

FTIR spectra showed several absorbance peaks in wavebands associated with bond vibrations in specific groups of compounds relevant for organic matter quality. These included peaks associated with C=C stretching (1620  $\text{cm}^{-1}$  and 1510  $\text{cm}^{-1}$ ) and C–O stretching (1420  $\text{cm}^{-1}$ ) in aromatic and carboxylic structures and C–O–C vibrations in polysaccharides (1020  $\text{cm}^{-1}$ ) (Artz et al., 2008; Haberhauer et al., 1998; Heller et al., 2015). Peak intensity for wavebands associated with aromatic compounds (sum of 1620  $\text{cm}^{-1}$ , 1510  $\text{cm}^{-1}$  and 1420  $\text{cm}^{-1}$ ) and polysaccharides (1020  $\text{cm}^{-1}$ ) was measured, whilst the aromaticity index was calculated as the ratio of absorbance in aromatic to polysaccharide wavebands (McAnallen et al., 2017).

## 2.5. Statistical analyses

All analyses were conducted using R statistical software (version 3.5.3) (R Development Core Team, 2017). To test for differences among plots in soil respiration, generalised linear mixed models (GLMMs) were used for repeated measurements, whilst generalised linear models (GLMs) were used to analyse data within time points. Prior to analysis, we tested for normality and skewness, subsequently using appropriate error distributions and link functions in the models (see Table S1 for full details). Tukey's HSD test was used for pairwise comparisons, conducted using the *multcomp* R package. The soil respiration measurements made on three consecutive days were averaged, with the mean value used in statistical analyses. Soil temperature can influence soil respiration rates (Schaefer et al., 2009), and was therefore included as a covariate in models analysing soil respiration. Soil moisture was not included as a covariate as it did not improve model fit, following comparison of Akaike information criterion (AIC) values. Cumulative respiration was calculated as the area under

the curve, with the log-transformed data analysed by one-way ANOVA followed by Tukey's HSD test for pairwise comparisons. One-way ANOVA and Tukey's HSD test was also used to compare the abundances of specific PLFAs associated with different microbial groups among plot types. Prior to analysis, PLFA abundance data for each microbial group were normalized by the total PLFA abundance to obtain relative abundance, correcting for varying extraction efficiency between samples. Concentration data for each individual PLFA were Hellinger transformed, centred and scaled prior to cross-validated principal components analysis (PCA) (Legendre and Gallagher, 2001; Legendre and Legendre, 1998). To statistically test for differences in the PLFA profiles of different plots, Euclidean distance matrices were analysed by permutational multivariate ANOVA (PERMANOVA) with 10000 permutations, using the *adonis* package. FTIR spectral data were pre-processed by baseline correction and smoothing using the *ChemoSpec* package, whilst the 1800-2500 cm<sup>-1</sup> region was removed to minimise background variability. Scaled and centred spectral data were visualised by PCA, and Euclidean distance matrices were analysed by PERMANOVA as previously described. FTIR peak intensities for the specific wavebands and ratios previously described were analysed in GLMs and GLMMs.

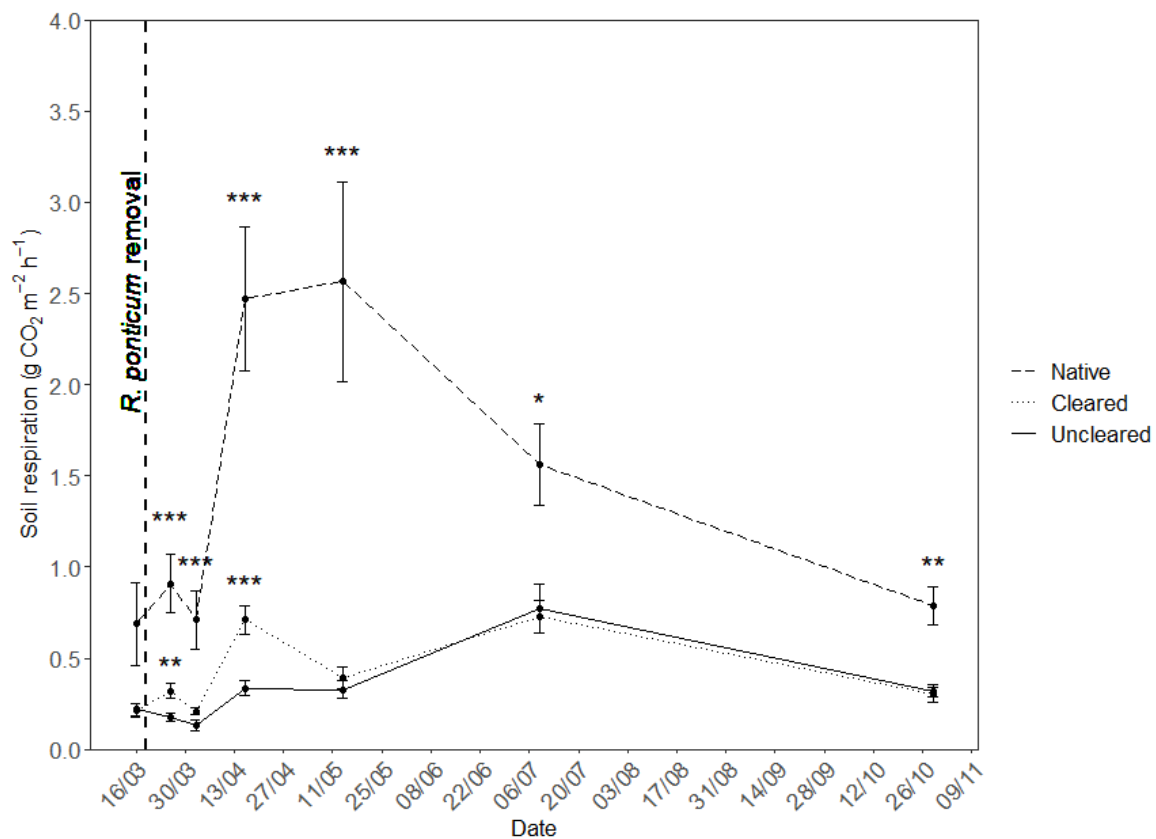
### **3. Results**

#### **3.1. Impact of *R. ponticum* on soil respiration**

Over the whole growing season, soil respiration was significantly lower in the uncleared plots relative to the native vegetation plots ( $P < 0.001$ ) (Figure 1). Soil temperature significantly affected soil respiration when included as a covariate in the above model ( $P < 0.001$ ) and was significantly higher in native vegetation plots relative to uncleared plots over the growing season ( $P < 0.001$ ) (Table S1). Soil moisture did not significantly differ among plot types ( $P = 0.170$ ), whilst PAR was significantly lower in uncleared plots ( $P < 0.001$ ) (Table S1).

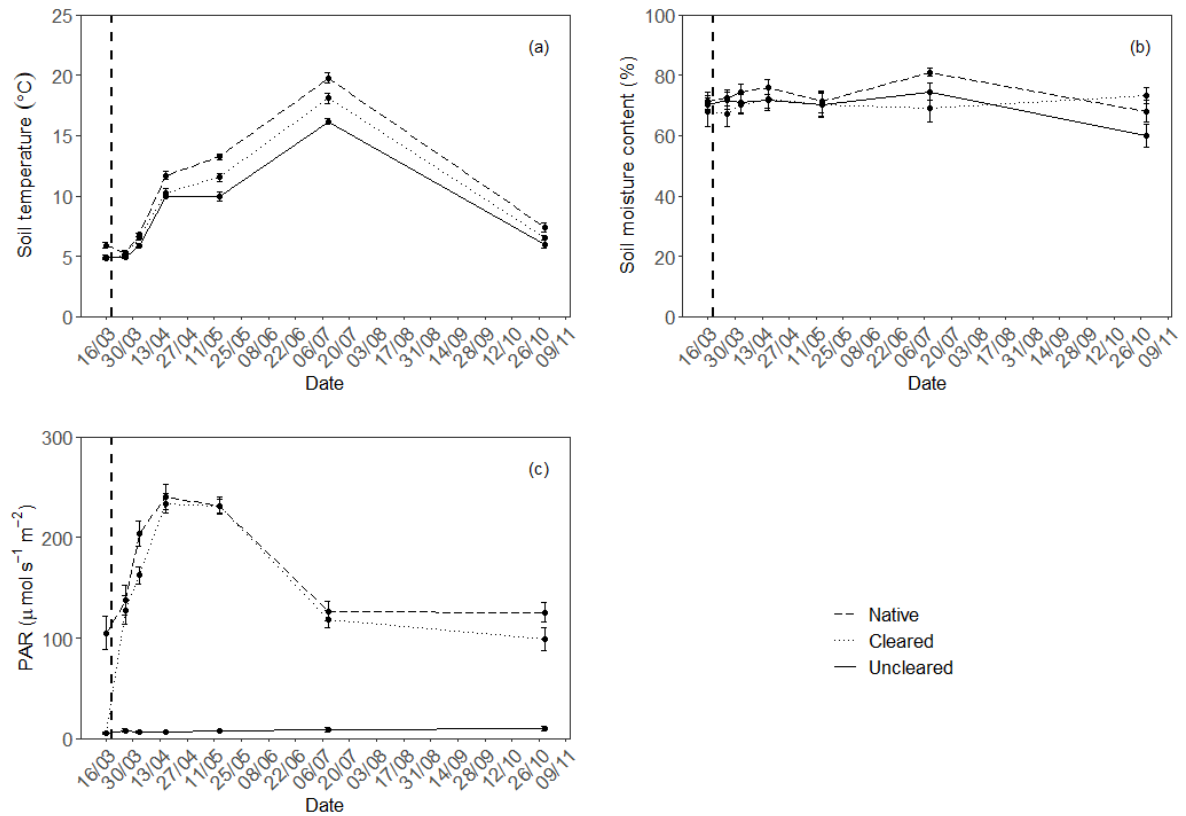
*R. ponticum* clearance did not increase soil respiration, with no significant difference in cumulative respiration between the cleared and uncleared plots over the growing season ( $P = 0.799$ ) (16.37 g

CO<sub>2</sub> m<sup>-2</sup> and 15.31 g CO<sub>2</sub> m<sup>-2</sup> respectively). Cumulative respiration during this period was significantly higher on the native vegetation plots (50.17 g CO<sub>2</sub> m<sup>-2</sup>), relative to the cleared and uncleared plots ( $P < 0.001$ ). Similarly, a repeated measures model detected no significant difference in soil respiration between the cleared and uncleared plots over the 32-week period post-clearance ( $P = 0.214$ ) (Figure 1). Soil respiration in the native vegetation plots remained significantly higher than in the cleared plots post *R. ponticum* removal over the same period ( $P < 0.001$ ) (Table S1). Repeated measures analysis detected soil temperature was significantly higher in cleared plots relative to uncleared plots over the 32 weeks post-clearance ( $P = 0.005$ ). When comparing plots within individual time points, higher soil respiration on cleared plots relative to uncleared plots was observed one- and four-weeks post-clearance ( $P = 0.009$  and  $P < 0.001$  respectively) (Figure 1). No differences were observed between these plots two ( $P = 0.222$ ), eight ( $P = 0.619$ ), 16 ( $P = 0.783$ ) or 32 weeks ( $P = 0.489$ ) post-clearance.



**Figure 1:** Mean soil respiration ( $\pm$  standard error) in the cleared, native vegetation and uncleared plots over the 32-week duration of the experiment. Plots were cleared of *R. ponticum* during the week beginning 19<sup>th</sup> March 2018. Significantly higher soil respiration in the cleared or native plots relative to the uncleared plots is denoted by \* ( $P < 0.05$ ), \*\* ( $P < 0.01$ ) and \*\*\* ( $P < 0.001$ ) following analysis in GLMs.

Despite soil respiration being lower in cleared and uncleared plots relative to native vegetation plots, no significant differences were detected in the soil carbon percentage of these plots prior to clearance ( $P = 0.315$ ), or 32 weeks post-clearance ( $P = 0.105$ ) (Table 1). Similarly, soil nitrogen percentage did not vary significantly among plot types prior to clearance ( $P = 0.084$ ) (Table 1). Cleared plots had significantly lower soil nitrogen percentage compared to the native plots 32 weeks post-clearance ( $P = 0.004$ ), but not compared to the uncleared plots ( $P = 0.176$ ) (Table 1). No significant differences in soil nitrogen percentage were detected between the uncleared and the native plots post-clearance ( $P = 0.293$ ) (Table 1). The soil C:N ratio of the uncleared plots was significantly higher than the native vegetation plots at the start of experiment ( $P = 0.026$ ), however no significant differences were detected among any of the plot types 32 weeks post-clearance ( $P = 0.139$ ). No significant differences in soil pH were observed among the different plot types either before or after clearance ( $P = 0.652$  and  $P = 0.749$  respectively) (Table 1).



**Figure 2:** Mean (a) soil temperature, (b) moisture content and (c) photosynthetically active radiation (PAR) ( $\pm$  standard error) in the cleared, native vegetation and uncleared plots over the 32-week duration of the experiment. The vertical dotted lines mark the time at which plots were cleared of *R. ponticum* (during the week beginning 19<sup>th</sup> March 2018).

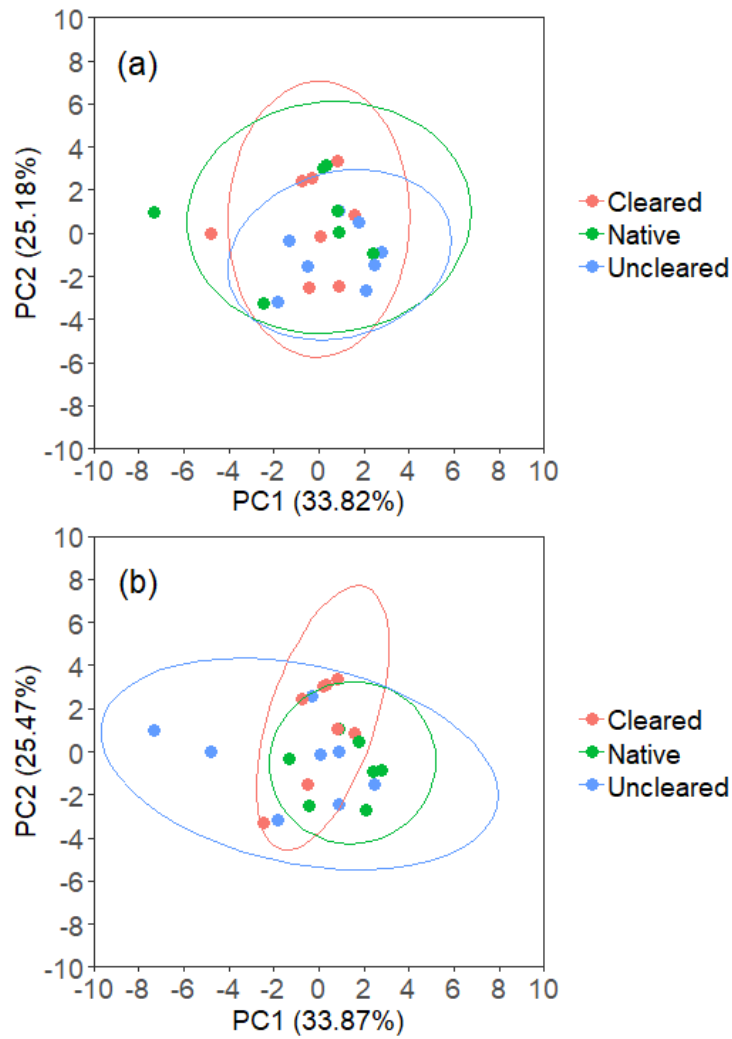
257 **Table 1:** Mean ( $\pm$  standard error) carbon content, nitrogen content, C:N ratio and pH of soils sampled from cleared, native vegetation or uncleared plots,  
 258 both before clearance and 32 weeks post-clearance. Common letters denote statistically non-significant differences ( $P > 0.05$ ) following analysis in GLMs.

	Prior to clearance			32 weeks post-clearance		
	Cleared	Native	Uncleared	Cleared	Native	Uncleared
C content (%)	25.27 $\pm$ 3.44	32.47 $\pm$ 3.27	28.62 $\pm$ 2.91	29.23 $\pm$ 3.28	37.13 $\pm$ 2.14	34.44 $\pm$ 1.97
N content (%)	1.35 $\pm$ 0.19	1.91 $\pm$ 0.16	1.51 $\pm$ 0.17	1.52 $\pm$ 0.17 a	2.14 $\pm$ 0.10 b	1.86 $\pm$ 0.12 ab
C:N	18.70 $\pm$ 0.55 ab	16.88 $\pm$ 0.71 a	19.31 $\pm$ 0.71 b	19.26 $\pm$ 0.94	17.26 $\pm$ 0.55	18.61 $\pm$ 0.50
pH	4.03 $\pm$ 0.07	4.09 $\pm$ 0.09	3.97 $\pm$ 0.08	3.83 $\pm$ 0.09	3.85 $\pm$ 0.04	3.89 $\pm$ 0.08

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### 3.2. Soil PLFA analysis

Hellinger transformed concentration data of 16 PLFAs extracted from soils collected prior to clearance and 32 weeks post-clearance were subjected to PCA. The first two PCs accounted for 59% of total variance prior to clearance, 59.34% post-clearance, and did not clearly separate the different plot types in either model (Figure 3). To statistically determine whether the clusters of each plot type were different, Euclidean distance matrices were analysed by PERMANOVA. These analyses corroborated the interpretation of the PCA, detecting no significant differences in PLFA profiles among the different plot types prior to clearance ( $P = 0.204$ ), or 32 weeks post-clearance ( $P = 0.066$ ). No significant differences in total PLFA concentration were observed among the cleared, uncleared and native plots either prior to clearance ( $P = 0.727$ ), or 32 weeks post-clearance ( $P = 0.128$ ) (Table 2). This was also true for the F:B and GP:GN ratios prior to clearance ( $P = 0.072$  and  $P = 0.942$  respectively) and 32-weeks post-clearance ( $P = 0.201$  and  $P = 0.758$ ). Prior to clearance, no significant differences in Gram-negative ( $P = 0.262$ ) or total bacterial ( $P = 0.085$ ) PLFA abundance were observed among plots. However, the plots that were to be cleared had significantly lower fungal PLFA abundance ( $P = 0.038$ ) and higher Gram-positive abundance ( $P = 0.029$ ) compared to the plots that would be left uncleared. No significant differences in the relative abundances of the different microbial groups were observed among plots post-clearance.



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278 **Figure 3:** Score plots for PCA models analysing the Hellinger transformed concentration data of the  
 279 16 PLFAs identified in the soil samples collected from the different plot types both (a) prior to *R.*  
 280 *ponticum* removal and (b) 32 weeks post-clearance. Plots were either cleared of *R. ponticum*, left  
 281 uncleared or uninvaded and consisting of native vegetation ( $n = 8$ ). Models were cross validated to  
 282 evaluate fit and avoid overfitting. Ellipses denote the 95% confidence intervals for each of the  
 283 different plot types.

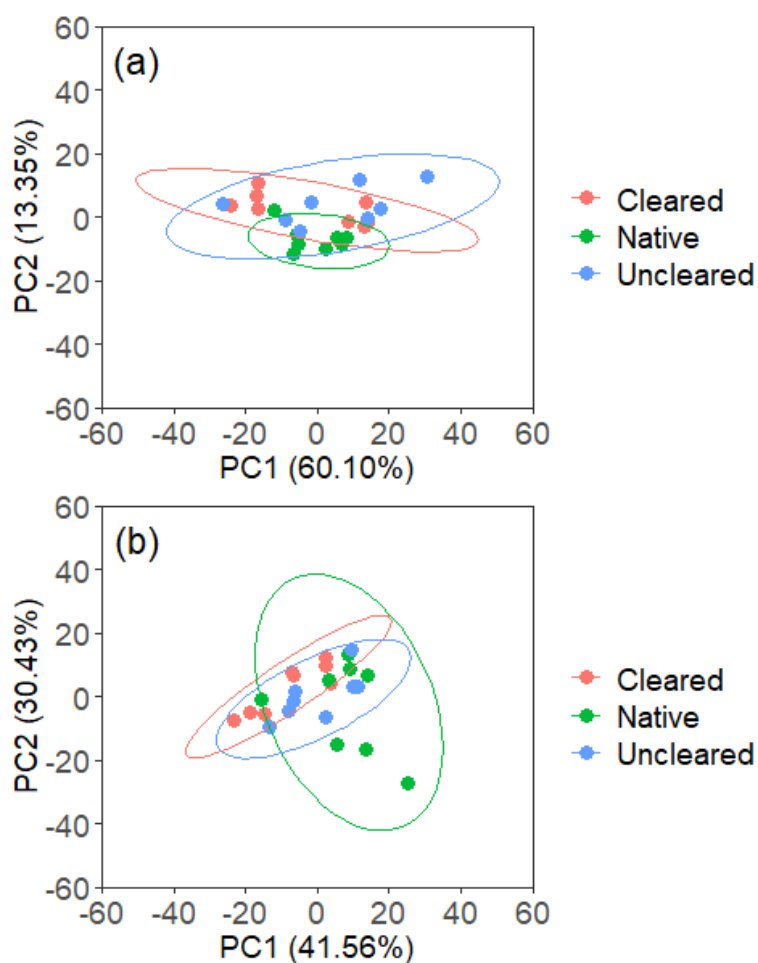


**Table 2:** Mean relative abundance (%) ( $\pm$  standard error) of PLFAs associated with different microbial groups in the samples collected from cleared, native vegetation or uncleared plots, both before clearance and 32 weeks post-clearance. Total PLFA concentration (nmol g<sup>-1</sup> soil) and the fungal: bacterial (F:B) and Gram-positive: Gram-negative (GP:GN) ratios are also given. Common letters denote non-significant differences among the different plot types following one-way ANOVA.

	Prior to clearance			32 weeks post-clearance		
	Cleared	Native	Uncleared	Cleared	Native	Uncleared
Fungal PLFAs (%)	44.14 $\pm$ 2.60 a	49.87 $\pm$ 1.80 ab	51.14 $\pm$ 0.99 b	51.89 $\pm$ 1.05	49.19 $\pm$ 1.26	50.48 $\pm$ 1.23
Bacterial PLFAs (%)	27.68 $\pm$ 1.58	25.04 $\pm$ 1.08	23.88 $\pm$ 0.66	26.65 $\pm$ 0.86	25.04 $\pm$ 0.98	21.92 $\pm$ 1.84
Gram-positive PLFAs (%)	14.25 $\pm$ 0.69 a	12.84 $\pm$ 0.52 ab	12.37 $\pm$ 0.25 b	11.86 $\pm$ 0.65	12.66 $\pm$ 0.60	11.25 $\pm$ 0.93
Gram-negative PLFAs (%)	13.02 $\pm$ 0.97	11.70 $\pm$ 0.71	11.17 $\pm$ 0.71	10.34 $\pm$ 0.41	11.91 $\pm$ 0.56	10.34 $\pm$ 0.11
Total PLFAs (nmol g <sup>-1</sup> soil)	146.13 $\pm$ 17.13	163.89 $\pm$ 14.40	141.82 $\pm$ 27.72	186.63 $\pm$ 33.72	212.78 $\pm$ 38.89	124.56 $\pm$ 8.26
F:B	1.66 $\pm$ 0.18	2.04 $\pm$ 0.16	2.16 $\pm$ 0.10	2.32 $\pm$ 0.12	2.00 $\pm$ 0.12	2.45 $\pm$ 0.25
GP:GN	1.12 $\pm$ 0.05	1.11 $\pm$ 0.05	1.14 $\pm$ 0.09	1.15 $\pm$ 0.07	1.07 $\pm$ 0.05	1.14 $\pm$ 0.12

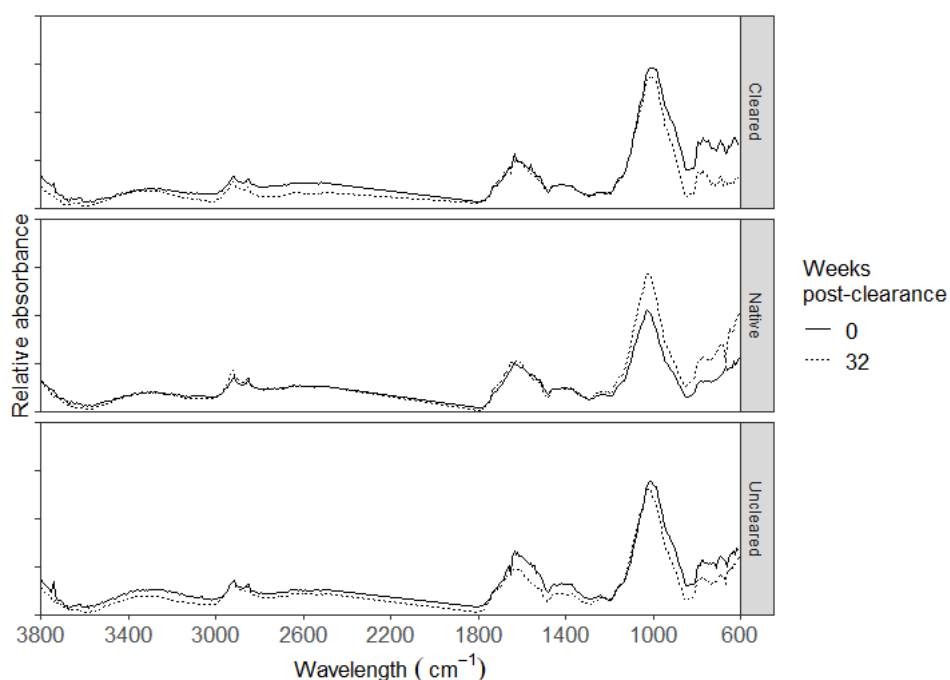
### 3.3. Soil FTIR spectra analysis

The first two PCs of soil samples collected from cleared, native vegetation and uncleared plots accounted for 73.45% of the total variance prior to clearance and 71.99% post-clearance. Partial separation by plot type was observed both prior to and 32 weeks post-clearance along PC1 and PC2 (Figure 4). However, PERMANOVA detected no significant differences in Euclidean distances among the different plot types, either prior to- or 32 weeks post-clearance ( $P = 0.056$  and  $P = 0.122$  respectively).



**Figure 4:** Score plots for PCA models analysing the FTIR spectra of soils collected from the different plot types both (a) prior to *R. ponticum* removal and (b) 32 weeks post-clearance. Plots were either cleared of *R. ponticum*, left uncleared or uninvaded and consisting of native vegetation ( $n = 8$ ). Models were cross validated to evaluate fit and avoid overfitting. Ellipses denote the 95% confidence intervals for each of the different plot types.

Visual inspection of FTIR spectra showed differences in peak intensities for specific wavebands relevant to SOM quality (Figure 5). At the start of the experiment, absorbance in the polysaccharide region was significantly lower for soil from native plots relative to the plots that would be cleared ( $P < 0.001$ ) and to the plots that would be left uncleared ( $P = 0.022$ ), whilst there was no difference between the cleared and uncleared plots ( $P = 0.541$ ) (Table 3). No differences among plot types were observed in this region post-clearance ( $P = 0.231$ ). Polysaccharide absorbance significantly increased for native plot soil over the 32-week growing season ( $P < 0.001$ ), whilst no significant change was observed for cleared and uncleared plots over the same period ( $P = 0.150$  and  $P = 0.917$  respectively). Absorbance in the region associated with aromatic structures did not vary among plots in samples collected either prior to or post-clearance ( $P = 0.197$  and  $P = 0.894$  respectively). Aromatic region absorbance significantly decreased over the growing season for uncleared plot soil ( $P = 0.049$ ), whilst no significant change was observed for cleared ( $P = 0.846$ ) or native ( $P = 0.975$ ) plot soil. The ratio of absorbance in aromatic to polysaccharide regions can be used as an index of aromaticity and humification (McAnallen et al., 2017). Soil aromaticity decreased over 32 weeks in both the native vegetation ( $P = 0.048$ ) and uncleared plots ( $P = 0.014$ ), however no change was observed for cleared plots ( $P = 0.957$ ).



**Figure 5:** Plot of FTIR spectra for soils sampled from plots cleared of *R. ponticum*, native vegetation plots and plots left uncleared. Soils were sampled prior to (0 weeks) and 32 weeks post-clearance.

**Table 3:** Mean ( $\pm$  standard error) relative absorption (measured as peak intensity) of soil FTIR spectra in wavebands associated with aromatic compounds ( $1620\text{ cm}^{-1}$ ,  $1510\text{ cm}^{-1}$  and  $1420\text{ cm}^{-1}$ ), polysaccharides ( $1020\text{ cm}^{-1}$ ), and the ratio of absorbance in aromatic to polysaccharide wavebands (aromaticity index). Soils were sampled both prior to and 32 weeks post *R. ponticum* clearance. Common letters denote statistically non-significant differences within plots over time.

Plot type	Weeks post-clearance	Waveband relative absorption		
		Aromatics	Polysaccharides	Aromaticity index
Cleared	0	$2.14 \pm 0.36$	$2.55 \pm 0.10$	$0.88 \pm 0.18$
	32	$2.06 \pm 0.26$	$2.39 \pm 0.13$	$0.89 \pm 0.13$
Native	0	$2.11 \pm 0.16$	$1.96 \pm 0.08\text{ a}$	$1.10 \pm 0.11\text{ a}$
	32	$2.11 \pm 0.24$	$2.63 \pm 0.12\text{ b}$	$0.81 \pm 0.10\text{ b}$
Uncleared	0	$2.76 \pm 0.45\text{ a}$	$2.38 \pm 0.14$	$1.15 \pm 0.16\text{ a}$
	32	$1.95 \pm 0.25\text{ b}$	$2.39 \pm 0.06$	$0.81 \pm 0.10\text{ b}$

#### 4. Discussion

Overall, soil respiration was significantly lower on uncleared *R. ponticum* plots relative to native vegetation plots, consistent with the first hypothesis. Soil moisture and temperature are both known to influence soil respiration rates (Hanson et al., 2000; Rutledge et al., 2010; Schaefer et al., 2009). However, only soil temperature contributed towards the variation in soil respiration between invaded and native vegetation plots in the current study, as soil moisture did not significantly vary among plot types. Soil temperature was consistently higher in native vegetation plots than in uncleared plots over the 32-week period monitored, most likely a consequence of the dense shade cast by the *R. ponticum* canopy. Despite the observed differences in soil respiration, soil carbon percentage did not vary between invaded and native plots. This may reflect the size of the soil

336 carbon pools, which are typically much larger than the amount of carbon released by soil respiration  
337 annually (Hartley et al., 2007; Valentini et al., 2000).

338 Leading from the lower soil respiration on uncleared plots relative to native vegetation plots, it was  
339 hypothesised that clearing *R. ponticum* would lead to increased soil respiration, relative to uncleared  
340 plots. Clearing led to a short-term increase in soil respiration at one- and four-weeks post-clearance.  
341 Several factors may have contributed towards this, including the observed increase in solar radiation  
342 at ground level post canopy removal, leading to higher soil temperature on cleared plots relative to  
343 uncleared plots, known to increase soil respiration (Rutledge et al., 2010; Yuste et al., 2004). Cutting  
344 woody shrubs and trees can also cause pulses in rhizodeposition and fine root mortality, which  
345 stimulate microbial activity and decomposition beneath the cut shrub (Frank et al., 2018; Pignataro  
346 et al., 2012).

347 The initial increase in soil respiration post-clearance did not persist over the whole growing season,  
348 despite the increase in soil temperature. This suggests that there were additional factors influencing  
349 soil respiration in cleared plot soil. Two explanations have previously been proposed for decreasing  
350 response in soil respiration to soil warming over time following perturbation (Hartley et al., 2007).

351 One potential reason is that the increase in soil temperature may cause a shift in soil microbial  
352 community structure, as different microbial groups have varying optimal temperature ranges  
353 (Hartley et al., 2007; Luo et al., 2001; Zogg et al., 1997). The altered microbial community may have  
354 access to a greater carbon pool, as they are able to metabolise carbon substrates that were  
355 unavailable to the community before soil warming (Zogg et al., 1997). This initially results in higher  
356 soil respiration, before declining over time as the community acclimates to the warmer soil  
357 temperature (Hartley et al., 2007; Luo et al., 2001; Zogg et al., 1997).

358 An altered microbial community is unlikely to explain the transient increase in soil respiration  
359 observed in the current study. Zogg et al. (1997) found shifts in PLFA profiles indicating altered  
360 microbial community structure in response to soil warming. However, we detected no significant

differences post-clearance among plot types in soil PLFAs, contrary to the third hypothesis, despite an increase in soil temperature post-clearance. The similar PLFA profiles of cleared, uncleared and native plot soils in the current study may reflect the low soil pH (pH of <4.1 for all plot types) and high C:N ratio of soil on the site, which tends to favour fungal dominated communities (Bååth and Anderson, 2003; Rousk et al., 2010). The PLFA method is regarded as an efficient and sensitive method of detecting shifts in the relative abundance of bacteria and fungi (Frostegård et al., 2011; Ramsey et al., 2006), and has detected shifts in previous studies on other invasives (Kourtev et al., 2003) and on soil warming (Zogg et al., 1997). Molecular techniques such as next generation sequencing may provide greater resolution for detecting finer changes in community composition, however Orwin et al. (2018) concluded that both approaches are broadly comparable. This is supported by the fact that no differences in soil F:B ratios were detected by Osburn et al. (2018) using a DNA sequencing technique two years post-clearance of the related *R. maximum*. Furthermore, the PLFA approach may be more suitable for detecting changes in higher taxonomic groups which lead to altered ecosystem functions (Orwin et al., 2018). We therefore consider the PLFA method to be appropriate for the current study.

Substrate limitation has also been suggested as a reason for declining soil respiration rates in response to increased soil temperature (Eliasson et al., 2005; Hartley et al., 2007). Soil warming initially leads to increased soil respiration, however microbial activity will decline over time as the pool of labile carbon becomes depleted (Eliasson et al., 2005; Hartley et al., 2007). This suggestion of substrate limitation is supported by the FTIR data, which is a commonly used technique to investigate shifts in SOM composition in a range of soil types (Elliott et al., 2007; Heller et al., 2015; McAnallen et al., 2017). Our fourth hypothesis which stated that soil FTIR spectra would be altered by clearing *R. ponticum* proved to be true; soil aromaticity was lower 32 weeks post-clearance (October 2018) compared to the start of the experiment (March 2018) in both the uncleared and native vegetation plots, whilst there was no change in this ratio in cleared plots during the same time period.

FTIR absorbance in regions associated with organic compounds may overlap with wavebands of mineral absorbance; for example  $\text{CaCO}_3$  absorbance may overlap with the  $1510\text{ cm}^{-1}$  and  $1620\text{ cm}^{-1}$  wavebands associated with aromatic organic compounds, whilst kaolinite absorbance may overlap with the polysaccharide waveband ( $1020\text{ cm}^{-1}$ ) (Le Guillou et al., 2015). Despite this, we consider our approach to be appropriate given the peaty nature of the Hexworthy soil (National Soil Resources Institute, 2019) and the igneous bedrock (British Geological Society, 2019) present on the site. As a result, the calcareous content of the soil would be low, thus the effect of overlapping  $\text{CaCO}_3$  absorbance is most likely negligible. Additionally, the low density of organic matter meant that by volume, the inorganic fraction of the soil was relatively low. Therefore, we argue that the soil organic fraction was the main influence on FTIR absorbance, and the overlapping of carbonates and clay minerals was likely to be negligible.

The fact that aromaticity decreased over the growing season for both uncleared and native plots, but not for the cleared plots suggests that *R. ponticum* removal influenced substrate quality. These observed variations in SOM quality may be due to changes in root exudation, canopy cover and litter deposition post-clearance. Whilst shrub clearance can lead to short-term pulses in rhizodeposition (Frank et al., 2018), canopy removal will result in an absence of photosynthesis in cleared plots, and thus lower photosynthate transfer to the soil in the longer-term, relative to uncleared and native vegetation plots. Additionally, carbon substrates are also introduced to the soil from canopy throughfall and in leachates from freshly deposited leaf litter (Frank et al., 2018; Hättenschwiler and Vitousek, 2000). The bare soil of the cleared plots would therefore likely have received lower input of fresh carbon substrates post removal of the *R. ponticum* canopy, relative to the soil of the uncleared and native plots. Thus, the pool of labile polysaccharides in the cleared plot soil would become depleted relative to the aromatic compounds, which are more resistant to decomposition and therefore become enriched in the soil (McAnallen et al., 2017; von Lützow et al., 2006).

411 Whilst soil aromaticity decreased for the uncleared plots and remained stable for the cleared plots  
412 over the experimental period, soil respiration did not differ at the end of this period between  
413 cleared and uncleared plots. This suggests that there were additional contributory factors  
414 influencing SOM decomposition in addition to substrate recalcitrance. Seasonal variation in soil  
415 temperature may be important, with the differences between cleared and uncleared plots becoming  
416 less pronounced in the Autumn. Soil physicochemical and biological factors are also known to  
417 influence decomposition, through organic matter stabilisation (Schmidt et al., 2011; von Lützow et  
418 al., 2006). Soil microbes may transform labile carbon substrates to stable compounds, which are  
419 more resistant to decomposition (Cotrufo et al., 2013; Schmidt et al., 2011). Carbon substrates may  
420 also adsorb to mineral surfaces or be physically occluded within soil aggregates, thus making them  
421 unavailable to decomposer organisms (Cotrufo et al., 2013; Schmidt et al., 2011; Zimmermann et al.,  
422 2012). Furthermore, carbon substrate availability to decomposer organisms can also be limited by  
423 binding to polyphenols (Bending and Read, 1997; Six et al., 2002). This may be particularly important  
424 for *R. ponticum*, as tannins leaching from the litter of the related *R. maximum* are reported to form  
425 decay-resistant complexes with SOM, leading to slower mineralisation rates (Wurzburger and  
426 Hendrick, 2009, 2007).

427 The lack of soil respiration response observed post-clearance in the current study may therefore be  
428 explained by substrate recalcitrance and SOM stabilisation. Differences in soil respiration remained  
429 between the cleared and native plots after one growing season. The persistence of this difference  
430 will likely depend on the revegetation of the bare soil left behind post-clearance, and a recent study  
431 highlighted that a native vegetation cover can take up to eight years to fully restore post *R. ponticum*  
432 removal (Jones et al., 2019). Therefore, to investigate whether soil processes and functioning in  
433 cleared plots transition towards native conditions post-clearance, a decadal study may be needed.



## 5. Conclusions

Our results show a short-term pulse in in soil respiration when *R. ponticum* is removed. This occurred during the initial four weeks post-clearance and was a response to higher soil temperature, but was limited in the longer-term by SOM quality. Soil PLFA profiles did not vary among cleared, native and uncleared plots post-clearance, indicating that *R. ponticum* removal did not alter the biomasses of different microbial groups. Our findings underline the strong influence of aboveground vegetation on soil respiration. Soil processes in the longer-term post-clearance will depend on the regeneration of native plant communities with cleared soil potentially taking decades to return to typical natural conditions.

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## References

- Aerts, R., 1999. Interspecific competition in natural plant communities: Mechanisms, trade-offs and plant-soil feedbacks. *Journal of Experimental Botany* 50, 29–37. doi:10.1093/jxb/50.330.29
- Aerts, R., 1997. Climate, leaf litter chemistry and leaf litter decomposition in terrestrial ecosystems: A triangular relationship. *Oikos* 79, 439–449. doi:10.2307/3546886
- Araujo, P.I., Austin, A.T., 2015. A shady business: Pine afforestation alters the primary controls on litter decomposition along a precipitation gradient in Patagonia, Argentina. *Journal of Ecology* 103, 1408–1420. doi:10.1111/1365-2745.12433

458 Artz, R.R.E., Chapman, S.J., Jean Robertson, A.H., Potts, J.M., Laggoun-Défarge, F., Gogo, S., Comont,  
 459 L., Disnar, J.R., Francez, A.J., 2008. FTIR spectroscopy can be used as a screening tool for  
 460 organic matter quality in regenerating cutover peatlands. *Soil Biology and Biochemistry* 40,  
 461 515–527. doi:10.1016/j.soilbio.2007.09.019

462 Austin, A.T., Méndez, M.S., Ballaré, C.L., 2016. Photodegradation alleviates the lignin bottleneck for  
 463 carbon turnover in terrestrial ecosystems. *Proceedings of the National Academy of Sciences*  
 464 113, 4392–4397. doi:10.1073/pnas.1516157113

465 Bååth, E., Anderson, T.H., 2003. Comparison of soil fungal/bacterial ratios in a pH gradient using  
 466 physiological and PLFA-based techniques. *Soil Biology and Biochemistry* 35, 955–963.  
 467 doi:10.1016/S0038-0717(03)00154-8

468 Bending, G.D., Read, D.J., 1997. Lignin and soluble phenolic degradation by ectomycorrhizal and  
 469 ericoid mycorrhizal fungi. *Mycological Research* 101, 1348–1354.  
 470 doi:10.1017/S0953756297004140

471 British Geological Society, 2019. Geology of Britain viewer [Web Document]. URL  
 472 <http://mapapps.bgs.ac.uk/geologyofbritain/home.html> (accessed 2.13.19).

473 Buyer, J.S., Sasser, M., 2012. High throughput phospholipid fatty acid analysis of soils. *Applied Soil*  
 474 *Ecology* 61, 127–130. doi:10.1016/j.apsoil.2012.06.005

475 Cotrufo, M.F., Wallenstein, M.D., Boot, C.M., Denef, K., Paul, E., 2013. The Microbial Efficiency-  
 476 Matrix Stabilization (MEMS) framework integrates plant litter decomposition with soil organic  
 477 matter stabilization: Do labile plant inputs form stable soil organic matter? *Global Change*  
 478 *Biology* 19, 988–995. doi:10.1111/gcb.12113

479 DeLuca, T.H., Zewdie, S.A., Zackrisson, O., Healey, J.R., Jones, D.L., 2013. Bracken fern (*Pteridium*  
 480 *aquilinum* L. kuhn) promotes an open nitrogen cycle in heathland soils. *Plant and Soil* 367, 521–  
 481 534. doi:10.1007/s11104-012-1484-0

482 Edwards, C., 2006. Managing and controlling invasive *Rhododendron*. Edinburgh.

483 Ehrenfeld, J.G., Kourtev, P., Huang, W., 2001. Changes in soil functions following invasions of exoctic  
484 understory indeciduous forests. *Ecological Applications* 11, 1287–1300. doi:10.2307/3060920

485 Eliasson, P.E., McMurtrie, R.E., Pepper, D.A., Strömgren, M., Linder, S., Ågren, G.I., 2005. The  
486 response of heterotrophic CO<sub>2</sub> flux to soil warming. *Global Change Biology* 11, 167–181.  
487 doi:10.1111/j.1365-2486.2004.00878.x

488 Elliott, G.N., Worgan, H., Broadhurst, D., Draper, J., Scullion, J., 2007. Soil differentiation using  
489 fingerprint Fourier transform infrared spectroscopy, chemometrics and genetic algorithm-  
490 based feature selection. *Soil Biology and Biochemistry* 39, 2888–2896.  
491 doi:10.1016/j.soilbio.2007.05.032

492 European Union, 2014. Regulation (EU) No 1143/2014 of the European Parliament and of the  
493 Council of 22 October 2014 on the prevention and management of the introduction and spread  
494 of invasive alien species.

495 Fanin, N., Kardol, P., Farrell, M., Nilsson, M.C., Gundale, M.J., Wardle, D.A., 2019. The ratio of Gram-  
496 positive to Gram-negative bacterial PLFA markers as an indicator of carbon availability in  
497 organic soils. *Soil Biology and Biochemistry* 128, 111–114. doi:10.1016/j.soilbio.2018.10.010

498 Frank, G.S., Nakatsu, C.H., Jenkins, M.A., 2018. Soil chemistry and microbial community functional  
499 responses to invasive shrub removal in mixed hardwood forests. *Applied Soil Ecology* 131, 75–  
500 88. doi:10.1016/j.apsoil.2018.08.005

501 Frostegård, Å., Tunlid, A., Bååth, E., 2011. Use and misuse of PLFA measurements in soils. *Soil*  
502 *Biology and Biochemistry* 43, 1621–1625. doi:10.1016/j.soilbio.2010.11.021

503 Galbraith, L., Wilkinson, S.G., 1991. Polar lipids and fatty acids of *Pseudomonas caryophylli*,  
504 *Pseudomonas gladioli* and *Pseudomonas pickettii*. *Journal of General Microbiology* 137, 197–  
505 202. doi:10.1016/j.camwa.2015.07.007

506 Gliksmann, D., Haenel, S., Osem, Y., Yakir, D., Zangy, E., Preisler, Y., Grünzweig, J.M., 2018. Litter  
 507 decomposition in Mediterranean pine forests is enhanced by reduced canopy cover. *Plant and*  
 508 *Soil* 422, 317–329. doi:10.1007/s11104-017-3366-y

509 Gliksmann, D., Rey, A., Seligmann, R., Dumbur, R., Sperling, O., Navon, Y., Haenel, S., De Angelis, P.,  
 510 Arnone, J.A., Grünzweig, J.M., 2017. Biotic degradation at night, abiotic degradation at day:  
 511 positive feedbacks on litter decomposition in drylands. *Global Change Biology* 23, 1564–1574.  
 512 doi:10.1111/gcb.13465

513 Haberhauer, G., Rafferty, B., Strebl, F., Gerzabek, M.H., 1998. Comparison of the composition of  
 514 forest soil litter derived from three different sites at various decompositional stages using FTIR  
 515 spectroscopy. *Geoderma* 83, 331–342. doi:10.1016/S0016-7061(98)00008-1

516 Hanson, P.J., Edwards, N.T., Garten, C.T., Andrews, J.A., 2000. Separating root and soil microbial  
 517 contributions to soil respiration: A review of methods and observations. *Biogeochemistry* 48,  
 518 115–146. doi:10.1023/A:1006244819642

519 Hartley, I.P., Heinemeyer, A., Ineson, P., 2007. Effects of three years of soil warming and shading on  
 520 the rate of soil respiration: Substrate availability and not thermal acclimation mediates  
 521 observed response. *Global Change Biology* 13, 1761–1770. doi:10.1111/j.1365-  
 522 2486.2007.01373.x

523 Hättenschwiler, S., Vitousek, P.M., 2000. The role of polyphenols in terrestrial ecosystem nutrient  
 524 cycling. *Trends in Ecology and Evolution*. doi:10.1016/S0169-5347(00)01861-9

525 Heller, C., Ellerbrock, R.H., Roßkopf, N., Klingenfuß, C., Zeitz, J., 2015. Soil organic matter  
 526 characterization of temperate peatland soil with FTIR-spectroscopy: Effects of mire type and  
 527 drainage intensity. *European Journal of Soil Science* 66, 847–858. doi:10.1111/ejss.12279

528 Hobbie, S.E., 1992. Effects of plant species on nutrient cycling. *Trends in Ecology and Evolution* 7,  
 529 336–339. doi:10.1016/0169-5347(92)90126-V

530 Horner, J.D., Gosz, J.R., Cates, R.G., 1988. The role of carbon-based plant secondary metabolites in  
 531 decomposition in terrestrial ecosystems. *The American Naturalist* 132, 869–883.  
 532 doi:10.1086/284894

533 Jackson, P., 2008. *Rhododendron* in Snowdonia and a strategy for its control.

534 Jewell, M.D., Shipley, B., Paquette, A., Messier, C., Reich, P.B., 2015. A traits-based test of the home-  
 535 field advantage in mixed-species tree litter decomposition. *Annals of Botany* 116, 781–788.  
 536 doi:10.1093/aob/mcv105

537 Jones, G.L., Tomlinson, M., Owen, R., Scullion, J., Winters, A., Jenkins, T., Ratcliffe, J., Gwynn-Jones,  
 538 D., 2019. Shrub establishment favoured and grass dominance reduced in acid heath grassland  
 539 systems cleared of invasive *Rhododendron ponticum*. *Scientific Reports* 9, 2239.  
 540 doi:10.1038/s41598-019-38573-z

541 Kidd Haack, S., Garchow, H., Odelson, D.A., Forney, L.J., Klug, M.J., 1994. Accuracy, reproducibility,  
 542 and interpretation of fatty acid methyl ester profiles of model bacterial communities. *Applied*  
 543 *and Environmental Microbiology* 60, 2483–2493. doi:10.1080/09638280802306638

544 Kourtev, P.S., Ehrenfeld, J.G., Häggblom, M., 2003. Experimental analysis of the effect of exotic and  
 545 native plant species on the structure and function of soil microbial communities. *Soil Biology*  
 546 *and Biochemistry* 35, 895–905. doi:10.1016/S0038-0717(03)00120-2

547 Le Guillou, F., Wetterlind, W., Viscarra Rossel, R.A., Hicks, W., Grundy, M., Tuomi, S., 2015. How does  
 548 grinding affect the mid-infrared spectra of soil and their multivariate calibrations to texture and  
 549 organic carbon? *Soil Research* 53, 913–921. doi:10.1071/SR15019

550 Lechevalier, H., Lechevalier, M.P., 1988. Chemotaxonomic use of lipids, in: *Microbial Lipids*.  
 551 Academic Press, pp. 869–902.

552 Legendre, P., Gallagher, E.D., 2001. Ecologically meaningful transformations for ordination of species  
 553 data. *Oecologia* 129, 271–280. doi:10.1007/s004420100716

554 Legendre, P., Legendre, L., 1998. Numerical Ecology, 2nd Englis. ed. Elsevier, Amsterdam.

555 Liao, C., Peng, R., Luo, Y., Zhou, X., Wu, X., Fang, C., Chen, J., Li, B., 2008. Altered ecosystem carbon  
556 and nitrogen cycles by plant invasion: A meta-analysis. *New Phytologist* 177, 706–714.  
557 doi:10.1111/j.1469-8137.2007.02290.x

558 Llorens, P., Domingo, F., 2007. Rainfall partitioning by vegetation under Mediterranean conditions. A  
559 review of studies in Europe. *Journal of Hydrology* 335, 37–54.  
560 doi:10.1016/J.JHYDROL.2006.10.032

561 Luo, Y., Wan, S., Hui, D., Wallace, L.L., 2001. Acclimatization of soil respiration to warming in a tall  
562 grass prairie. *Nature* 413, 622–625. doi:10.1038/35098065

563 McAnallen, L., Doherty, R., Ogle, N., 2017. Organic composition and multiphase stable isotope  
564 analysis of active, degrading and restored blanket bog. *Science of the Total Environment* 599–  
565 600, 1779–1790. doi:10.1016/j.scitotenv.2017.05.064

566 Met Office, 2018. Met Office Integrated Data Archive System (MIDAS).

567 Monk, K., Bass, D., Brown, N.D., Hemery, G.E., 2014. Post-clearance effects of rhododendron on the  
568 fungal communities of the eastern sidelands of Lundy, Bristol Channel. *Journal of the Lundy*  
569 *Field Society* 4, 57–70.

570 Moorhead, D.L., Callaghan, T., 1994. Effects of increasing ultraviolet B radiation on decomposition  
571 and soil organic matter dynamics: a synthesis and modelling study. *Biology and Fertility of Soils*  
572 18, 19–26. doi:10.1007/BF00336439

573 National Soil Resources Institute, 2019. Soil Site Report for location 269079E 344069N, 5km x 5km.  
574 Cranfield, UK.

575 Orwin, K.H., Dickie, I.A., Holdaway, R., Wood, J.R., 2018. A comparison of the ability of PLFA and 16S  
576 rRNA gene metabarcoding to resolve soil community change and predict ecosystem functions.

577        Soil Biology and Biochemistry 117, 27–35. doi:10.1016/j.soilbio.2017.10.036

578        Osburn, E.D., Elliott, K.J., Knoepp, J.D., Miniat, C.F., Barrett, J.E., 2018. Soil microbial response to  
579        Rhododendron understory removal in southern Appalachian forests: Effects on extracellular  
580        enzymes. Soil Biology and Biochemistry 127, 50–59. doi:10.1016/J.SOILBIO.2018.09.008

581        Pattison, Z., Rumble, H., Tanner, R.A., Jin, L., Gange, A.C., 2016. Positive plant-soil feedbacks of the  
582        invasive *Impatiens glandulifera* and their effects on above-ground microbial communities.  
583        Weed Research 56, 198–207. doi:10.1111/wre.12200

584        Pignataro, A., Moscatelli, M.C., Mocali, S., Grego, S., Benedetti, A., 2012. Assessment of soil  
585        microbial functional diversity in a coppiced forest system. Applied Soil Ecology 62, 115–123.  
586        doi:10.1016/j.apsoil.2012.07.007

587        Plocher, A.E., Carvell, K.L., 1987. Population dynamics of rosebay rhododendron thickets in the  
588        southern Appalachians. Bulletin of the Torrey Botanical Club 114, 121–126.

589        R Development Core Team, 2017. R: A language and environment for statistical computing. R  
590        Foundation for Statistical Computing, Vienna, Austria.

591        Ramsey, P.W., Rillig, M.C., Feris, K.P., Holben, W.E., Gannon, J.E., 2006. Choice of methods for soil  
592        microbial community analysis: PLFA maximizes power compared to CLPP and PCR-based  
593        approaches. Pedobiologia 50, 275–280. doi:10.1016/j.pedobi.2006.03.003

594        Ratledge, C., Wilkinson, S.G., 1988. Microbial Lipids. Academic Press, London.

595        Rousk, J., Baath, E., Brookes, P., Lauber, C.L., Lozupone, C., Caporaso, J.C., Knight, R., Fierer, N., 2010.  
596        Soil bacterial and fungal communities across a pH gradient in an arable soil. Applied and  
597        Environmental Microbiology 4, 1340–1351. doi:10.1128/AEM.01476-12

598        Rutledge, S., Campbell, D.I., Baldocchi, D., Schipper, L.A., 2010. Photodegradation leads to increased  
599        carbon dioxide losses from terrestrial organic matter. Global Change Biology 16, 3065–3074.

doi:10.1111/j.1365-2486.2009.02149.x

Schaefer, D.A., Feng, W., Zou, X., 2009. Plant carbon inputs and environmental factors strongly affect soil respiration in a subtropical forest of southwestern China. *Soil Biology and Biochemistry* 41, 1000–1007. doi:10.1016/j.soilbio.2008.11.015

Schmidt, M.W.I., Torn, M.S., Abiven, S., Dittmar, T., Guggenberger, G., Janssens, I.A., Kleber, M., Kögel-Knabner, I., Lehmann, J., Manning, D.A.C., Nannipieri, P., Rasse, D.P., Weiner, S., Trumbore, S.E., 2011. Persistence of soil organic matter as an ecosystem property. *Nature* 478, 49–56. doi:10.1038/nature10386

Six, J., Conant, R.T., Paul, E.A., Paustian, K., 2002. Stabilization mechanisms of soil organic matter: Implications for C-saturation of soils. *Plant and Soil*. doi:10.1023/A:1016125726789

Snowdonia Rhododendron Partnership, 2015. The Ecosystem Benefits of managing the invasive non-native plant *Rhododendron ponticum* in Snowdonia Snowdonia Rhododendron Partnership 2015 Rhododendron in Snowdonia.

Suseela, V., Alpert, P., Nakatsu, C.H., Armstrong, A., Tharayil, N., 2016. Plant–soil interactions regulate the identity of soil carbon in invaded ecosystems: Implication for legacy effects. *Functional Ecology* 30, 1227–1238. doi:10.1111/1365-2435.12591

Tamura, M., Suseela, V., Simpson, M., Powell, B., Tharayil, N., 2017. Plant litter chemistry alters the content and composition of organic carbon associated with soil mineral and aggregate fractions in invaded ecosystems. *Global Change Biology* 23, 4002–4018. doi:10.1111/gcb.13751

Tamura, M., Tharayil, N., 2014. Plant litter chemistry and microbial priming regulate the accrual, composition and stability of soil carbon in invaded ecosystems. *New Phytologist* 203, 110–124. doi:10.1111/nph.12795

Tyler, C., Pullin, A.S., Stewart, G.B., 2006. Effectiveness of management interventions to control invasion by *Rhododendron ponticum*. *Environmental Management*. doi:10.1007/s00267-005-



624 0127-0

625 Valentini, R., Matteucci, G., Dolman, A.J., Schulze, E.D., Rebmann, C., Moors, E.J., Granier, A., Gross,  
626 P., Jensen, N.O., Pilegaard, K., Lindroth, A., Grelle, A., Bernhofer, C., Grünwald, T., Aubinet, M.,  
627 Ceulemans, R., Kowalski, A.S., Vesala, T., Rannik, Ü., Berbigier, P., Loustau, D., Guomundsson, J.,  
628 Thorgeirsson, H., Ibrom, A., Morgenstern, K., Clement, R., Moncrieff, J., Montagnani, L.,  
629 Minerbi, S., Jarvis, P.G., 2000. Respiration as the main determinant of carbon balance in  
630 European forests. *Nature* 404, 861–865. doi:10.1038/35009084

631 Van Der Heijden, M.G.A., Bardgett, R.D., Van Straalen, N.M., 2008. The unseen majority: Soil  
632 microbes as drivers of plant diversity and productivity in terrestrial ecosystems. *Ecology*  
633 *Letters*. doi:10.1111/j.1461-0248.2007.01139.x

634 von Lützow, M., Kögel-Knabner, I., Ekschmitt, K., Matzner, E., Guggenberger, G., Marschner, B.,  
635 Flessa, H., 2006. Stabilization of organic matter in temperate soils: Mechanisms and their  
636 relevance under different soil conditions - A review. *European Journal of Soil Science*.  
637 doi:10.1111/j.1365-2389.2006.00809.x

638 Wurzburger, N., Hendrick, R.L., 2009. Plant litter chemistry and mycorrhizal roots promote a  
639 nitrogen feedback in a temperate forest. *Journal of Ecology* 97, 528–536. doi:10.1111/j.1365-  
640 2745.2009.01487.x

641 Wurzburger, N., Hendrick, R.L., 2007. Rhododendron thickets alter N cycling and soil extracellular  
642 enzyme activities in southern Appalachian hardwood forests. *Pedobiologia* 50, 563–576.  
643 doi:10.1016/j.pedobi.2006.10.001

644 Xiao, H., Meissner, R., Seeger, J., Rupp, H., Borg, H., 2009. Effect of vegetation type and growth stage  
645 on dewfall, determined with high precision weighing lysimeters at a site in northern Germany.  
646 *Journal of Hydrology* 377, 43–49. doi:10.1016/j.jhydrol.2009.08.006

647 Yuste, J.C., Janssens, I.A., Carrara, A., Ceulemans, R., 2004. Annual Q10 of soil respiration reflects

648 plant phenological patterns as well as temperature sensitivity. *Global Change Biology* 10, 161–  
649 169. doi:10.1111/j.1529-8817.2003.00727.x

650 Zelles, L., 1999. Fatty acid patterns of phospholipids and lipopolysaccharides in the characterisation  
651 of microbial communities in soil: A review. *Biology and Fertility of Soils*.  
652 doi:10.1007/s003740050533

653 Zimmermann, M., Leifeld, J., Conen, F., Bird, M.I., Meir, P., 2012. Can composition and physical  
654 protection of soil organic matter explain soil respiration temperature sensitivity?  
655 *Biogeochemistry* 107, 423–436. doi:10.1007/s10533-010-9562-y

656 Zogg, G.P., Zak, D.R., Ringelberg, D.B., White, D.C., MacDonald, N.W., Pregitzer, K.S., 1997.  
657 Compositional and functional Shifts in Microbial Communities Due to Soil Warming. *Soil*  
658 *Science Society of America Journal* 61, 475. doi:10.2136/sssaj1997.03615995006100020015x

659